effect of tetrapropylammonium (TPA) and Beryllium Fluoride (BeFx) on inward proton current (IH) transport by NKA expressed in Xenopus oocytes. TPA (a shared site blocker) had a dual effect on IH; between pH 7.6-6.0 TPA partially inhibited IH, and at pH 5.0 (where an inhibitory effect on the steady state IH is present), TPA partially activated IH. When the phosphate analogue BeFx was injected into oocytes, stabilizing an externally open conformation of the ion binding sites, K^+ still inhibited inward current at pH 7.6. At pH 5.0 K^+ activated IH resembling the effect of TPA on fully functional NKA. These results illustrate the complex interaction between the occupancy of shared sites and proton permeation. Blocking access at the shared sites with TPA activated IH by relieving the inhibition normally observed at high $[H^+]$. Partial inhibition by TPA at pH 5.0 may reflect incomplete block of H^+ access or a reduced rate of the conformational changes needed for H^+ translocation. After BeFx inhibition NKA was still capable of interacting with K^+. The mixed occupancy of K^+ and H^+ at low pH activated the leak, likely relieving the H^+ inhibition due to K^+ occlusion. Supported by NIH GM 061583 to CG and NSF MCB-1243842 to PA.

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Potassium Regulation of the NaK-ATPase Pump Currents in Mammalian Skeletal Muscle Fibers

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Adult skeletal muscles express two Na,K-ATPase isoforms, α1 and α2. α2, the sole isoform expressed in the transverse tubules, comprises up to 90% of total Na,K-ATPase. α2 in resting muscle operates well below its maximum turnover rate; but is rapidly stimulated, by yet unknown mechanisms, during muscle contraction, helping maintaining excitation and resist fatigue. We tested the hypothesis that α2 activity may be regulated by extracellular K over the physiological range of K concentrations that occur in the t-tubules. Na,K-ATPase driven current (Ipump) was measured in enzymatically isolated FDB fibers voltage clamped at −90 mV using a two microlectrode amplifier. Ipump was identified as a ouabain- and temperature-sensitive outward current activated by Ko in fibers rendered electrically passive. Intracellular solutions (filling both electrodes) contained (mM): 1EGTA, 0.5CaCl2, 5MgCl2, 20MOPS, 5glu-thathion-H2, 90K-asparte, and 5ATP-Na2, 5-phosphocreatine-Na2, and 30Na-aspartate to promote forward pump cycling. External solutions contained (mM): 2CaCl2, 1MgCl2, 10glucose, 10MOPS; and inversely varying amounts of KCl (0-40) and NaCL (154-114) to maintain osmolarity. TTX (400nM), nifedipine (20 μM), Ba (1mM) and 9-anthracene-carboxilic-acid (200μM) were added to block main ionic currents. All solutions had pH=7.4 and 300mOmal/kgH2O. Ipump was activated in a concentration-dependent manner by raising [K]o from 0 to 40mM, and eliminated by washing out K. Ipump was prevented by pretreatment with 10μM ouabain or inhibited by addition of ouabain, and was steeply temperature-dependent. Maximal Ipump vs. [K]o was fitted to obtain the Km for pump activation by K. Results demonstrate that α2 in skeletal muscle is regulated by changes in extracellular K over tens of mM, suggesting that this isoform is adapted to respond to expected demands of Na/K transport in the t-tubules during sustained activity. (Supported by the National institutes of Health, USA)

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A Combination of Curcumin with Either Gramicidin or Ouabain Selectively Kills Cells that Express the Multidrug Resistance-Linked ABCG2 Transporter

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The work presented here introduces a strategy to kill selectively multidrug resistant cells that express the ABCG2 transporter (also called breast cancer resistance protein, BCRP). The approach is based on specific stimulation of ATP hydrolysis by ABCG2 transporters with sub-toxic doses of curcumin combined with stimulation of ATP hydrolysis by the Na^+ K^+ ATPase with sub-toxic doses of gramicidin A or ouabain. After 72 h of incubation with the drug combinations, the resulting overconsumption of ATP by both pathways inhibits the efflux activity of ABCG2 transporters, leads to depletion of intracellular ATP levels below the viability threshold, and kills resistant cells selectively over cells that lack ABCG2 transporters. This strategy, which was also tested on a clinically relevant human breast adenocarcinoma cell line (MCF-7/FLV1), exploits the overexpression of ABCG2 transporters and induces caspase-dependent apoptotic cell death selectively in resistant cells. This work thus introduces a novel strategy to exploit collateral sensitivity (CS) with a combination of two clinically used compounds that, individually, do not exert CS. Collectively, this work expands current knowledge on ABCG2-mediated CS and provides a potential strategy for discovery of CS drugs against drug-resistant cancer cells.

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Conformational Changes and Complex Formation of the Non-Canonical Ribose ABC Transporter

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Bacterial ATP-Binding Cassette (ABC) transporters are vital for nutrient uptake. Structural and functional studies have revealed two distinct types (I and II) of importers based primarily upon mechanistic differences. The ribose transporter (RbsABC) in E. coli is a tripartite ABC importer. RbsABC consists of a cytoplasmic ATP-binding cassette, RbsA, with dual fused nucleotide-binding domains (NBD), a homodimeric transmembrane domain (TMD), RbsC, and a periplasmic substrate binding protein (SBP), RbsB. This study, using Electron Paramagnetic Resonance (EPR) spectroscopy and biochemical methods, demonstrates that RbsABC is a non-canonical ABC transporter that shares some structural and functional features with both type I and type II importers. ATP hydrolysis in the NBD is stimulated by the presence of substrate-loaded SBP, leading to ribose release, which is typical of type I importers. Quantitative measurements obtained from site-directed spin-labeling EPR spectroscopy reveal that hydrolysis promotes the opening of RbsB to release ribose and subsequently RbsB. Post-hydrolysis, both nucleotide and magnesium are required to maintain the complex interactions of RbsC and RbsA, based upon co-purification studies and EPR experiments, which is atypical for ABC importers of either type, where the high-affinity TMD-NBD association is maintained perpetually. In addition, the presence of ribose has a destabilizing effect on RbsB-RbsC interactions, a type II importer behavior. Further, RbsB and RbsC interact in an unusual apo-complex consisting only of the SBP-TMD. Finally, substrate transport is accomplished by hydrolysis at a single consensus site in RbsA, with the second site rendered degenerate by mutations of conserved amino acids. This feature is not uncommon to ABC exporters, but heretofore unobserved in importers. Taking these observations together, RbsABC appears to transport substrate by a distinct mechanism, and offers an opportunity to gain insight into the function of non-canonical systems.

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Movement of the Nucleotide Binding Domains in the ABC Transporter MsbA Reconstituted in Nanodiscs

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MsbA is a homodimeric bacterial lipid flippase homolog of P-glycoprotein, an ABC- transporter involved in multidrug resistance. ABC transporters are formed by two transmembrane domains and two highly conserved nucleotide binding domains (NBDs) that bind and hydrolyze ATP. Crystal structures have shown widely separated NBDs (open conformation; nucleotide-free state) and NBDs forming a dimer with nucleotide trapped at the interface (closed conformation, nucleotide-bound), leading to the proposal of a switch model, where NBDs associate/dissociate during the ATP hydrolysis cycle. Other proposed mechanisms suggest instead that the NBDs are always in contact. Recent studies using Luminescence Resonance Energy Transfer (LRET) in a detergent-solubilized single-cysteine MsbA mutant (T561C; Cooper & Altenberg, 2013. JBC 287:14994) have shown transitions between open (52 Å) and closed NBD conformations (36 Å), with the proportion of molecules in each conformation changing during the hydrolysis cycle: majority open in nucleotide-free, majority closed in nucleotide-bound, ~50% each during hydrolysis. Now, we have used LRET to determine if this large NBD separation occurs when MsbA is reconstituted in a membrane bilayer. MsbA (T561C) was labeled with LRET probes, was reconstituted in nanodiscs, and the distance changes were determined in nucleotide-free, nucleotide-bound, and hydrolysis conditions (MgATP). We found that the largest distance observed for the reconstituted MsbA was 46 Å (partially-open conformation), suggesting smaller conformational changes than in detergent. We also found that, opposite to what happens in detergent, only about half of the molecules adopt the ''open'' conformation in the nucleotide-free condition, indicating that the membrane stabilizes the closed conformation. These results show an important effect of the lipid bilayer, and suggest small conformational changes during the hydrolysis cycle of MsbA under near ''physiological'' conditions. This work was supported by CPRIT grant RP101073.